

Effects of Aerobically Oxidized Cellulose on Blood Coagulation¹ (36483)

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Purified cotton cellulose after burning in air is often mentioned in the oriental countries as a home remedy to stop bleeding of minor cuts. The present report is concerned with the effects of aerobically oxidized cellulose on blood coagulation.

Materials and Methods. *Preparation of aerobically oxidized cotton (AOC).* Purified cotton cellulose (surgical cotton) was burned in air under a hood. As soon as flames had subsided the combustion was stopped by transferring the charred material to a round-bottom flask and refluxed for 6 hr with water. The aqueous extract was concentrated in vacuum on a flash evaporator. The concentrate was then centrifuged at 40,000 rpm for 1 hr in a preparative ultracentrifuge to remove all the finely divided suspended material. The clear supernatant was lyophilized in a freeze-drier to a light-yellow powder. The preparation (AOC) used throughout the present study was a 60% alcohol precipitated material obtained from an aqueous solution of the lyophilized product after adjusting the pH to 7.0. Five pounds of cotton cellulose yielded 200 mg AOC. No attempt was made at this time to further purify AOC. Hydrolysis of AOC by 3% H₂SO₄ for 90-120 min at 100° released glucose and glucuronic acid in the ratio of 1:1. Glucose was determined by Somogyi's method while glucuronic acid was measured by the procedure of Bitter. The presence of these two compounds and traces of 2-ketogluconic acid in the hydrolysate was confirmed by ascending paper chromatographic procedure using *n*-propanol:ethylacetate:H₂O in ratios of 7:1:2 by volume as the developing solvent and ammoniacal AgNO₃ was used as the detection agent.

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Procedure for studying clotting properties of recalcified plasma and whole-blood. Blood was drawn in silicone-treated syringes and used as such or was mixed with 9 vol blood to 1 vol 3.8% trisodium citrate. Plasma was obtained by centrifugation of the citrated blood at 2500 rpm (1000g) for 20 min. It was stored at 4° and used within a 1 week period. Pyrex glassware or glass syringes were siliconized by rinsing twice with "Siliclad" (obtained from Clay Adams Inc., NY 10010) and then heating to 160° for 1 hr in an oven. Whole blood clotting time was measured on 1 ml of blood alone or containing 5 mg cellulose oxidized by nitrogen dioxide or 0.1 mg AOC in siliconized Pyrex test tubes (130 × 10 mm). Clotting was allowed to proceed at room temperature (20°). At half-minute intervals the tubes were tilted and the clotting time recorded when the tubes could be inverted without spilling the blood. Clotting time of recalcified plasma was measured in an essentially similar manner. Three-tenths of a milliliter of the plasma was mixed with 0.3 ml 0.1 M imidazole buffer pH 7.3. To this mixture was added 0.3 ml 0.025 M CaCl₂ and the timing was started. Six clotting time determinations were made and the means and the standard deviations were calculated. The pretreatment of plasma with glass, "Celite" or adsorption by BaSO₄ was carried out by the procedure of Nossel. The procedure for testing the clotting times of the treated plasma was the same as above except when the BaSO₄ adsorbed plasma (0.3 ml) was added to the "Celite"-exhausted plasma (0.3 ml), the concentration of the CaCl₂ (0.3 ml) used was doubled to 0.05 M.

Studies were conducted on the effect of AOC on the clotting time of recalcified plasma where the reaction mixture included one of the clot-promoting agents *vis.* tissue thromboplastin, or thrombin or Russell's

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TABLE I. Effect of Aerobically Oxidized Cellulose (AOC) on Whole Blood Clotting Time of Different Animals in Siliconized Tubes.

| Compound added | Species | | |
|--|----------------------|---------------|----------------|
| | Human | Rat | Rabbit |
| | Clotting time in min | | |
| None | 26.0 \pm 1.5 | 9.5 \pm 1.0 | 23.0 \pm 1.4 |
| AOC | 7.0 \pm 0.7 | 3.0 \pm 0.5 | 6.0 \pm 0.6 |
| Cellulose oxidized by nitrogen dioxide | 17.0 \pm 1.1 | 8.0 \pm 0.8 | 21.0 \pm 0.8 |

viper venom (obtained from Calbiochem, Los Angeles, CA 90063). The test mixture for this study was the same as before except for these additions. The latter agents are reported to enhance clotting by mechanisms which do not require the activation of the Hageman factor. AOC was tested for its effect in reversing the inhibition of clotting by heparin and in this experiment thrombin, tissue thromboplastin and Russell's viper venom were compared with AOC in reversing the heparin inhibition.

Results and Discussion. Table I compares the effect of cellulose oxidized by nitrogen dioxide with the material obtained by aerobic degradation (AOC) on the clotting time of whole blood obtained from different species. Cellulose oxidized by nitrogen dioxide is not soluble in the reaction mixture while AOC gives a clear tan-colored solution. The results show the clot-promoting effect of AOC in the three species tested while the cellulose oxidized by nitrogen dioxide was only slightly effective in human blood. Cellulose oxidized by nitrogen dioxide is available com-

mercially for use as a topical hemostatic agent in the forms, Oxycel (Parke Davis & Co., Detroit, MI) and Surgicel (Johnson & Johnson, New Brunswick, NJ). This material contains up to 25% of the primary hydroxyl groups of the cellulose oxidized to carboxyl groups. It is evident from the analytical figures that AOC resembles but is not identical with these commercial forms of oxidized cellulose. In this experiment blood clotting was tested in siliconized tubes and therefore the intrinsic pathway was mainly concerned in the clotting sequence. Comparison of different silicone-coated materials for clot-delaying properties is reported to show differences in effectiveness (1). Therefore it is important to use the same product throughout the study and a direct comparison of results using different products can not be made.

In order to determine the site of action of AOC, its effect on the clotting time of recalcified plasma was tested. The results of this study presented in Table II show that AOC enhances clotting in siliconized glass tubes. On the other hand, the clotting times

TABLE II. Effect of AOC on the Clotting Time of Glass Contacted or "Celite"-Exhausted or BaSO₄ Adsorbed Plasma in Siliconized Tubes.

| Plasma | AOC 30 μ g | Control |
|---|----------------------|----------------|
| | Clotting time in min | |
| Untreated | 7.0 \pm 0.8 | 12.0 \pm 0.9 |
| Glass contacted | 6.0 \pm 0.7 | 6.0 \pm 0.8 |
| "Celite" exhausted | 13.0 \pm 0.9 | 13.0 \pm 0.8 |
| "Celite" exhausted + BaSO ₄ adsorbed plasma heated at 56° for 30 min | 10.0 \pm 0.7 | 13.0 \pm 0.6 |
| "Celite" exhausted + BaSO ₄ adsorbed plasma heated at 65° for 15 min | 29.0 \pm 1.1 | 29.0 \pm 1.0 |

TABLE III. Effect of AOC on the Later Stages of Clotting in Siliconized Tubes.

| Clot-promoting agent | AOC 30 μ g | Heparin 0.25 μ g | Control |
|------------------------------------|-------------------|-------------------------|----------------|
| Clotting time in min | | | |
| None | 8.0 \pm 0.8 | 30.0 \pm 1.5 | 12.0 \pm 0.9 |
| Thrombin, 1 mg | 7.0 \pm 0.7 | 7.0 \pm 0.9 | 7.0 \pm 0.8 |
| Tissue thromboplastin, 0.02 mg | 6.0 \pm 0.8 | 27.0 \pm 1.4 | 7.0 \pm 0.7 |
| Russell's viper venom, 0.2 μ g | 6.0 \pm 0.5 | 29.0 \pm 2.4 | 8.0 \pm 0.6 |
| AOC, 30 μ g | | 7.0 \pm 0.7 | |

of untreated plasma in plain tubes with or without the addition of AOC was 7.0 ± 0.7 . This would indicate that AOC activated Hageman factor, in a manner analogous to the glass surface (2). AOC did not accelerate the clotting of glass-contacted plasma because the Hageman and PTA factors have already been activated by contact with the glass surface. "Celite"-exhausted plasma gave similar clotting times with or without AOC because "Celite" treatment is reported to preferentially remove part of the Hageman and PTA factors (3). On the other hand, BaSO_4 treatment and heating at 56° for 30 min is reported to remove most of the clotting factors other than Hageman and PTA factors and therefore this plasma accelerates clotting when added to "Celite"-exhausted plasma. Heating the BaSO_4 -treated plasma to 65° for 15 min destroys the Hageman and PTA factors and therefore the AOC acceleration is not observed when this plasma is added to "Celite"-exhausted plasma (Table II). These results give further evidence that AOC may promote clotting by activating the Hageman and the PTA factors.

The results of Table III show that AOC has little or no effect on the clot-promoting effect of thrombin, tissue thromboplastin or Russell's viper venom indicating that its effect may be localized to the early steps in the coagulation of the recalcified plasma. Effect of AOC on heparin inhibition of the coagulation process was tested because of the possible similarity in the structural backbone between AOC and heparin. The results of this study also presented in Table III show that AOC effectively reverses the inhibition by heparin. Under these experimental conditions, Russell's viper venom or tissue throm-

boplastin were without effect while thrombin at 1 mg levels reversed the inhibition. Heparin is reported (4) to have no inhibitory effect on the surface activation by Hageman factor, but the activity of "activated" PTA is inhibited by heparin. Therefore it is possible that at the levels tested the inhibitory effect of heparin is mainly on the early phases of clotting through the inhibition of PTA. AOC may be reversing this effect by activating the Hageman factor which in turn activates the PTA and the clotting sequence continues. It appears from the above results that the reversal of heparin inhibition by AOC is not due to specific antagonistic effects at different sites in the clotting system, but is the result of a direct competition analogous to a metabolite-inhibitor relationship. Studies are in progress to further purify and characterize AOC and until these are completed it can not be speculated whether this is the case.

Summary. Aerobically oxidized cellulose (AOC) accelerates clotting time of whole blood from human, rabbit and rat in siliconized tubes. Studies of its effect on the clotting sequence of recalcified human plasma show that AOC may activate the Hageman and PTA factors in a manner similar to glass surface. Heparin inhibition of clotting of recalcified plasma was effectively reversed by AOC. This may be due to an inhibitor-metabolite relationship between heparin and AOC.

1. Fisher, L. M., and Jaques, L. B., *J. Appl. Physiol.* 13, 527 (1958).
2. Ratnoff, O. D., and Rosenblum, J. M., *Amer. J. Med.* 25, 160 (1958).
3. Margolis, J., *J. Physiol.* 137, 95 (1957).
4. Bloom, A. L., *J. Clin. Pathol.* 15, 508 (1962).

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